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**Annex 1. Maintenance logbook**

**Annex 2. Fluorescence microscope log-sheet**

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1. Scope

This SOP describes the optimal operation of a fluorescence microscope through regular servicing and preventive maintenance.

1. Definitions and abbreviations

*microscope magnification*

individual objective magnification x eyepiece magnification

NA: not applicable n n

1. Personnel qualifications
2. Medical fitness

In accordance with national laws and practices, arrangements should be made for appropriate health surveillance of TB laboratory workers:

* before enrolment in the TB laboratory;
* at regular intervals thereafter, annually or bi-annually;
* after any biohazard incident;
* at the onset of TB symptoms.

Ideally, individual medical records shall be kept for up to 10 years following the end of occupational exposure.

Laboratory workers should be educated about the symptoms of TB and provided with ready access to free medical care if symptoms arise.

Confidential HIV counselling and testing should be offered to laboratory workers. Options for reassignment of HIV-positive workers away from the high-risk environment of the TB laboratory should be considered.

All cases of disease or death identified in accordance with national laws and/or practice as resulting from occupational exposure to biological agents shall be notified to the competent authority.

1. Education and training

Education and training must be given on the following topics:

* potential risks to health (symptoms of TB disease and transmission);
* precautions to be taken to minimize aerosol formation and prevent exposure;
* hygiene requirements;
* wearing and use of protective equipment and clothing;
* handling of potentially infectious materials;
* laboratory design, including airflow conditions;
* prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards);
* good laboratory practice;
* organization of work flow;
* use of equipment (operation, identification of malfunctions, maintenance).

The training shall be:

* given before a staff member takes up his/her post;
* strictly supervised;
* adapted to take account of new or changed conditions; and
* repeated periodically, preferably every year.
1. Procedure

4.1 Principle

The fluorescence microscope is a precision instrument intended for microscopic detection of tubercle bacilli in specimens in the routine diagnostic TB laboratory.

The use of fluorochromes for staining allows smear examination at lower magnification than is used for light microscopy. Because of the lower magnification, each field examined under fluorescence microscopy is larger in area than that seen with light microscopy, thus reducing the time needed to examine a slide.

High-volume laboratories, dealing with more than 25 slides per day, could use a fluorescence microscope.

4.2 Samples

NA

4.3 Equipment and materials

A binocular microscope equipped with a fluorescent light source and suitable filter set for auramine-stained smears. Fluorescent light is provided by a mercury vapour lamp, a halogen lamp or a light-emitting diode (LED). The mercury vapour lamp provides the strongest light but it has a limited life of about 100-200 hours, which must be monitored with a timer.

Modern fluorescence microscopes do not require a completely dark room – turning off the light and covering the windows with curtains is usually sufficient – although darker conditions may be necessary for halogen-lamp fluorescence microscopes. In hot climates, the room should be permanently air conditioned, both for operator comfort and to protect the microscope from fungal growth when humidity is high.

Do not place the microscope where it could be exposed to direct sunlight, dust, vibration (e.g. from centrifuges), water (sink, spray from a tap), chemical reagents, or humidity.

Install the microscope on a rigid, flat, level surface. It is too large and sensitive to be moved regularly; thus, any protection from humidity or dust that is needed should be provided for the microscope in situ.

4.4 Reagents and solutions

Cleaning fluid, as recommended by the manufacturer, or 80/20 ethylether/alcohol or 70% alcohol.

Lens paper, muslin or silk cloth, or fine-quality toilet paper to clean lenses without scratching.

Microscope cover, plastic or cloth.

Immersion oil, if needed (does not have to be special non-fluorescing quality).

4.5 Detailed instructions for use

See SOP on auramine staining

4.6 Reading and recording

NA

4.7 Quality control and maintenance

*4.7.1 General maintenance*

The fluorescence microscope requires careful maintenance from both optical and mechanical points of view. Laboratory workers must be familiar with its general mechanical and optical principles. Record maintenance in the logbook (Annex 1).

* A mercury vapour lamp has a life of 100–200 hours and should be replaced after 100 hours of use. This time can be slightly exceeded, but the risk of the lamp exploding increases. Lamps from different manufacturers will have different lamp lifespans. They are expensive, so it is important to check the lifespan of each lamp and ensure that it is not exceeded (Annex 2).
* It may be possible to repair a faulty microscope by replacing easily removable parts (objectives, eyepieces, light bulbs, fuses); if this does not work, the microscope should be entrusted to a competent person for repair. *Never dismantle the microscope* – its operational maintenance efficiency and accuracy may be severely impaired.
* In climates with relative humidity in excess of 70% for more than just a few weeks a year, fungal growth may damage the microscope. Fungal growth occurs almost exclusively on the prisms in the binocular tube, causing haziness and then dimness, and finally obscuring the view completely. Check the microscope for fungal growth from time to time and whenever the view gets hazy. With the light on and the 10x objective in place, fungal growth can be seen easily by removing the eyepieces and looking into the binocular tube.

Fungal growth is best removed by a trained person. The binocular tube must be opened but the prisms must remain exactly as fixed by the factory: taking them out would destroy the microscope.

* The correct procedure for changing mercury lamps must be used, by trained technicians:
* do not touch the lamp with the fingers;
* check which end should point downwards (described on package insert) and respect this way of mounting;
* adjust the position (horizontal and vertical) of the new lamp and of the lamp-house mirror by using the adjustment knobs or refer to the microscope manual; replacement of halogen lamps does not require these adjustments.
* If the blue light remains weak after a new lamp has been fitted and properly adjusted, there may be another problem such as a blackened heat filter. Discontinue use of the microscope and request repair by a specialist.

*4.7.2 Daily maintenance.*

* In a humid climate without continuous air conditioning and if the microscope has no dedicated antifungal protection (a special device inserted under the binocular tube), stand a dish of silica gel (250g) on the microscope stage and cover the microscope with a plastic cover. Renew the silica gel whenever it turns pink, which may be daily; regenerate the gel by heating until it turns blue again.
* Check for broken or damaged parts
* Check the counter to ensure that the lifespan of the lamp has not been exceeded.
* Check the lenses for dirt or grit; they may easily becomes scratched if they are wiped without first blowing away dust and small sand particles. First blow the lens clean, using a blower brush if available, then clean the lenses with clean, dry lens paper (or suitable equivalent). If this does not produce a clear image, try again using the cleaning fluid provided by the manufacturer, or 80/20 ethylether/alcohol or 70% alcohol on the tissue paper.
* It is best not to remove eyepieces or objectives from their fixation holes but to clean only their external surfaces as needed. For proper cleaning of its lower lens, the condenser may have to be removed from its fixing. When replacing the condenser, ensure that slides moving over it cannot scratch its upper surface with the condenser in the uppermost position.
* It is equally important to make sure that holes for the eyepieces and objectives are never left open for more than a few minutes. If a lens is missing lens, close the fixation hole using the plug provided or by sticking adhesive tape over it, otherwise dust will enter and cause haziness of the remaining objectives.

*4.7.3 Monthly maintenance*

* Blow dust off the lenses, using a blower brush if available, before cleaning them. Then apply cleaning fluid (or suitable equivalent), NOT xylene) to the lens paper (or suitable equivalent) and clean the lenses.
* Remove the slide holder from the mechanical stage and clean it in the same way.
* Wipe dust off the body of the microscope with soft tissue paper moistened with water.

*4.7.4 Yearly maintenance*

* Thorough inspection and service by a qualified service technician.

4.8 Waste management and other safety precautions

Worn or broken mercury vapour lamps should be disposed off as toxic waste. In case of explosion, leave the room immediately and arrange for thorough air change before entering again –*mercury vapour is toxic*.

1. Related documents

Manufacturer’s manual, specific to each microscope.

*Basics of quality assurance for intermediate and peripheral laboratories*, 2nd ed. Cairo, WHO Regional Office for the Eastern Mediterranean, 2002.

Kent PT, Kubica GP. *Public health mycobacteriology: a guide for the level III laboratory*. Atlanta, GA, United States Department of Health and Human Services, Centers for Disease Control, 1985.

*Laboratory services in tuberculosis control. Part II: Microscopy*. Geneva, World Health Organization, 1998 (WHO/TB/98.258).

Lumb R, Bastian I. *Laboratory diagnosis of tuberculosis by sputum microscopy*. Adelaide, Institute of Medical and Veterinary Science, 2005.

*Maintenance and repair of laboratory, diagnostic imaging and hospital equipment*. Geneva, World Health Organization, 1994.

*Maintenance manual for laboratory equipment*, 2nd ed. Geneva, World Health Organization, 2008 (available at www.who.int/entity/diagnostics\_laboratory/documents/guidance/guidance2/en/)

*Manual of basic techniques for a health laboratory*, 2nd ed. Geneva, World Health Organization, 2003.

Rieder HL et al. *Priorities for tuberculosis bacteriology services in low-income countries,* 2nd ed. Paris, International Union Against Tuberculosis and Lung Disease, 2007.

**Annex 1. Maintenance logbook**

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| **ITEM IDENTIFICATION** |
| Equipment: MICROSCOPE | Brand name: |
| Purchase date: | Model/type: |
| Location within the laboratory: | Serial no. |
| Warranty expiry date: |
| Manufacturer: | Tel: |
| Address:. |
| Contact person: |
| Technical service representative: | Tel: |

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|  **PERIODICITY:**  |
| Date | Maintenance operation | Operator |
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| **FAILURE EVENTS** |
| Date | Event  | Corrective action taken | Operator |
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**Annex 2. Fluorescence microscope log-sheet**

The mercury vapour lamp has a life of approximately 100-200 hours. Replace the lamp after 100 hours of use

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| Operator's name | Date | Time | Duration of use (hours) | Cumulative duration of use (hours) |
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